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# Regulation of mRNA Decay by Puf Proteins is Dependent on Environmental Conditions

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# Regulation of mRNA Decay by Puf Proteins is Dependent on Environmental Conditions

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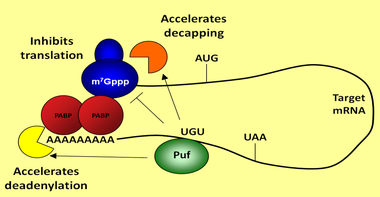
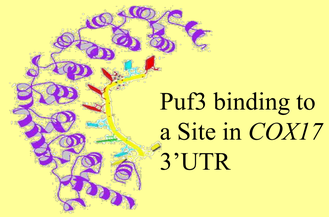
## Abstract

The nucleus of a eukaryotic cell contains the genetic code within DNA that directs growth and function of the cell. The genes contained in DNA make copies of themselves called RNAs, which are molecules able to leave the nucleus and direct protein synthesis. Over- or under-production of any one protein can cause cell malfunction and disease. Regulation of RNA lifespan is one method to ensure proper protein production. The Puf family of RNA-binding proteins regulate mRNA lifespans by controlling the rate of mRNA decay. More specifically, Puf proteins stimulate the removal of the poly(A) tails of mRNAs, which results in translational inhibition of the mRNA into protein and leads to complete mRNA degradation. In yeast, the Puf3 protein (Puf3p) regulates hundreds of mRNAs that encode proteins necessary for mitochondrial function. The ability of Puf3p to regulate mRNA decay is altered by the type of sugar source present. Puf3p is turned "on" in the presence of galactose and "off" in dextrose. The goal of this study was to analyze the effects of mutations in a component of the RNA decay machinery (Pop2p) that is required for condition-specific Puf3-mediated decay stimulation. The mutational effects were tested on both an mRNA normally targeted for degradation by Puf3p (COX17) and a control mRNA not targeted for decay (CBS1). Transcriptional shut-offs were performed to examine the decay rate of pooled mRNA following inhibition of mRNA production. When the temperature sensitive yeast cells are heat-shocked, new mRNA production is turned off and the existing mRNAs begin to decay over time. If the Pop2p mutations affect the decay rate of COX17 only, a regulation mechanism specific to Puf3-mediated decay is indicated. If both COX17 and CBS1 decay rates are affected, a global RNA-decay control mechanism is indicated.

## Background

**Puf Proteins Regulate mRNA Targets by Binding to Specific UGU-Containing Sequences in the 3' Untranslated Region (3' UTR) of the mRNA, Recruiting mRNA Decay Machinery, and Blocking Translation Initiation**

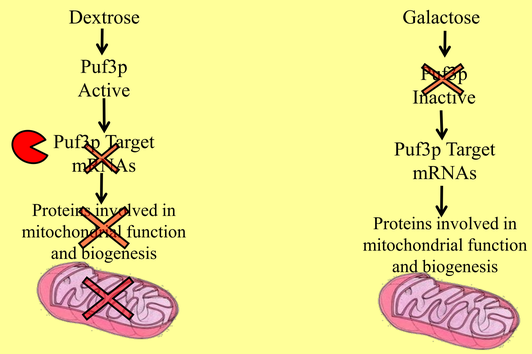


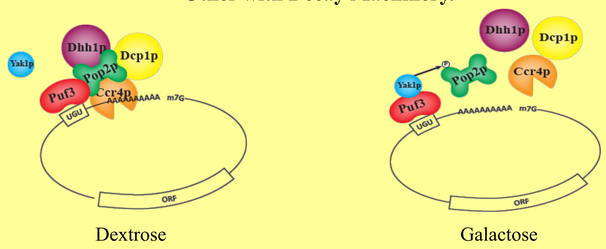
Puf3 binding to a Site in *COX17* 3'UTR

Zhu, Deyu et al. "A 5' Cytosine Binding Pocket in Puf3p Specifies Regulation of Mitochondrial mRNAs." *Proceedings of the National Academy of Sciences of the United States of America* 106.48 (2009): 20192–20197. *PMC*. Web. 26 Apr. 2017.

**Puf3p Activity Reflects a Stress Response and Mitochondrial Response**




**Phosphorylation of Pop2p at Thr97 Alters Decay Rates of mRNAs by Influencing Binding Affinity to Puf3p, and May Influence Pop2p Interactions with Other with Decay Machinery.**




**What is the role of Yak1?**

- Yak1: protein kinase that phosphorylates Pop2 at Thr97
  - We hypothesize that this phosphorylation decreases interaction between Puf3p and Pop2, and thereby stabilizes mRNA
- Previously determined that deletion of Yak1 allows rapid decay of Puf3 target RNAs in galactose.

**Research Questions:**

- Is phosphorylation of Pop2 part of a regulatory mechanism for decay of Puf3 target mRNAs, or is it a global mechanism for regulating decay of all mRNAs?
- How do mutations that alter Pop2 phosphorylation affect decay rate of COX17 (Puf3 target) and CBS1 (non-target) mRNAs in dextrose versus galactose conditions?

**Approach:**

- Mutate Thr97 of Pop2 to Ala (T97A) to block phosphorylation. Mutate Thr97 of Pop2 to Asp (T97D). The negative charge on Asp mimics a constant phosphorylated state of Pop2.
- Compare mRNA decay rates between COX17 (Puf3 target) and CBS1 (nuclear encoded mitochondrial mRNA, non-Puf3 target) by the two Pop2 mutants (T97A and T97D).

**Hypothesis:**

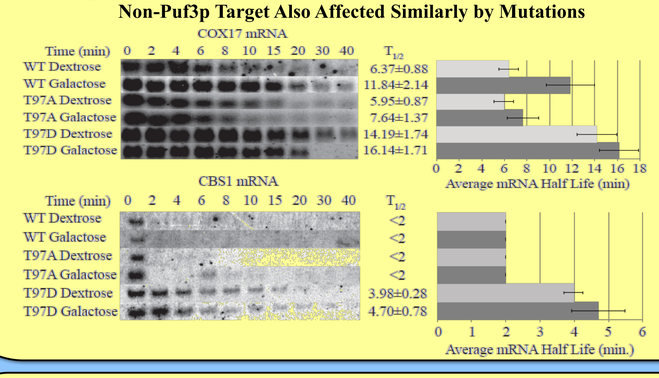
- When Pop2 is mutated to a state that mimics constitutive phosphorylation, Puf3 action will be inhibited, mRNA will stabilize, and mRNA decay half-lives will increase.
- When Pop2 is mutated to block phosphorylation, Puf3 action will be constitutive in both carbon sources, and mRNAs will decay rapidly with a short half-life.

## Methods

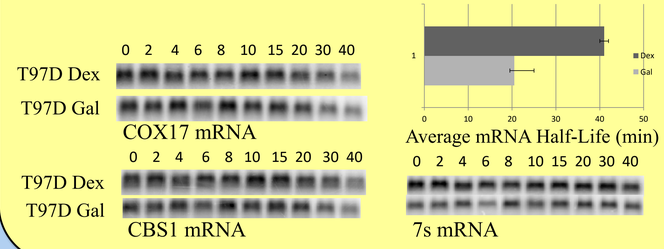
- WT, T97A, and T97D yeast strains were cultured overnight in media containing either galactose or dextrose at 25°C.
- **Transcription Shutoffs:** Cells were temperature shocked at 37°C to cause mRNA transcription shutoff. The cells were collected at time points 0, 2, 4, 6, 8, 10, 15, 20, 30, and 40 minutes.
- **Hot Phenol RNA Extraction:** RNA was extracted from each time point, quantified, and dried to 40ng/µl.
- **Northern Blots:** RNA was separated on 1% agarose gels and transferred to membranes. RNA was then crosslinked to membranes.
- **Probes:**
  - The membranes were incubated with a radioactive probe specific to COX17, scanned, quantified with ImageQuant.
  - The COX17 probe was removed and the CBS1 probe was added. Membranes were then quantified and normalized to the 7S control RNA signal.

## Results

**A Pop2p T97A (Phosphoblock) Mutation Enhances Decay of Puf3p Target Transcript, While a Pop2p T97D (Phosphomimetic) Mutation Stabilizes Puf3p Target Transcript Non-Puf3p Target Also Affected Similarly by Mutations**




**Transcriptional Shut-Off Assays for Endogenous T97D mutant mRNA Half-Life Analyses Support Hypothesis and Past Findings**




## Conclusions & Future Work

**Conclusions:**

- Results support hypothesis that a constitutively phosphorylated Pop2 mimic stabilizes mRNA by increasing the half-life of mRNAs in both dextrose and galactose as compared to wild type Pop2.
- Results support conclusion that Pop2's influence on mRNA decay is a global mechanism in mRNA regulation because both COX17 and CBS1 were affected.

**Future directions:**

- Replicate experiment with endogenous T97A mutant to corroborate results.